

WHAT IS CLAIMED IS:

1. An *in vitro* protein or nucleic acid synthesis system comprising one or more components selected from the group consisting of:

at least one extract from a cell having reduced activity of at least one enzyme that catalyzes hydrolysis of high energy phosphate bonds or hydrolysis or formation of phosphodiester bonds;

at least one inhibitor of at least one enzyme that catalyzes hydrolysis of high energy phosphate bonds or hydrolysis or formation of phosphodiester bonds; and

at least two energy sources providing chemical energy for synthesis.

2. The *in vitro* synthesis system according to claim 1, wherein the at least one extract from a cell has reduced activity of at least one nuclease.

3. The *in vitro* synthesis system according to claim 1, wherein the at least one extract from a cell has reduced activity of at least one phosphatase.

4. The *in vitro* synthesis system according to claim 1, wherein the at least one extract from a cell has reduced activity of at least one polymerase.

5. The *in vitro* synthesis system according to claim 1, wherein the at least one inhibitor inhibits at least one nuclease.

6. The *in vitro* synthesis system according to claim 1, wherein the at least one inhibitor inhibits at least one phosphatase.

7. The *in vitro* synthesis system according to claim 1, wherein the at least one inhibitor inhibits at least one polymerase.

8. The *in vitro* synthesis system according to claim 2, wherein the template is a DNA template and the nuclease is a DNase.

9. The *in vitro* synthesis system according to claim 8, wherein the DNase is a DNA exonuclease.

10. The *in vitro* synthesis system according to claim 8, wherein the DNase is a DNA endonuclease.

11. The *in vitro* synthesis system according to claim 10, wherein the DNA endonuclease is endonuclease A.

12. The *in vitro* synthesis system according to claim 2, wherein the nuclease is an RNase.

13. The *in vitro* synthesis system according to claim 12, wherein the RNase is an RNA exonuclease.

14. The *in vitro* synthesis system according to claim 12, wherein the RNase is an RNA endonuclease.

15. The *in vitro* synthesis system according to claim 14, wherein the endonuclease is RNase E.

16. The *in vitro* synthesis system according to claim 1, further comprising at least one nucleic acid template selected from the group consisting of a DNA template and an RNA template.

17. The *in vitro* synthesis system according to claim 16, comprising at least one DNA template and wherein the *in vitro* synthesis system is an *in vitro* transcription/translation system.

18. The *in vitro* synthesis system according to claim 3, wherein the phosphatase is an alkaline phosphatase.

19. The *in vitro* synthesis system according to claim 1, wherein the at least two energy sources generate or regenerate high energy triphosphate compounds.

20. The *in vitro* synthesis system according to claim 1, comprising at least one or more compounds selected from the group consisting of pyruvate, phosphoenolpyruvate (PEP), carbamoyl phosphate, acetyl phosphate, creatine phosphate, phosphopyruvate, glyceraldehyde-3-phosphate and glucose-6-phosphate.

21. The *in vitro* synthesis system according to claim 1, wherein the at least one extract from said cell is reduced in activity of at least one enzyme selected from the group consisting of OmpT, RNase E, alkaline phosphatase and endonuclease I.

22. The *in vitro* synthesis system according to claim 21, further reduced in at least one activity selected for the group consisting of RNase I or RNase I\*.

23. The *in vitro* synthesis system according to claim 1 comprising at least two energy sources providing chemical energy for synthesis.

24. The *in vitro* synthesis system according to claim 23, further comprising at least one extract from a cell having reduced activity of at least

one enzyme selected from the group consisting of a nuclease, a polymerase and a phosphatase.

25. The *in vitro* synthesis system according to claim 23, further comprising at least one inhibitor of at least one enzyme selected from the group consisting of a nuclease, a polymerase and a phosphatase.

26. The *in vitro* synthesis system according to claim 23, further comprising at least one inhibitor of at least one enzyme selected from the group consisting of a nuclease, a phosphatase and a polymerase.

27. The *in vitro* synthesis system according to claim 1, wherein the at least one enzyme is selected from RecBCD and the at least one inhibitor is at least Gam.

28. The *in vitro* synthesis system according to claim 1, wherein the at least one inhibitor is at least a soluble Gam.

29. An *in vitro* synthesis system according to claim 1 comprising one or more nucleic acid templates and one or more components selected from the group consisting of:

at least one inhibitor of an enzyme that degrades said template;

and

at least one extract of a cell having reduced degradative effect on said template.

30. The *in vitro* synthesis system according to claim 29, comprising at least one energy source.

31. The *in vitro* synthesis system according to claim 23, wherein the at least one energy source comprises at least two different energy sources,

each of which generates or regenerates high energy triphosphate compounds for the synthesis.

32. The *in vitro* synthesis system according to claim 31, wherein the at least two different chemical fuel sources are selected from the group consisting of pyruvate, phosphoenolpyruvate (PEP), carbamoyl phosphate, acetyl phosphate, creatine phosphate, phosphopyruvate, glyceraldehyde-3-phosphate and glucose-6-phosphate.

33. The *in vitro* synthesis system according to claim 32, wherein the at least two different chemical fuel sources comprise at least PEP and acetyl phosphate.

34. The *in vitro* synthesis system according to claim 1, comprising said at least one extract, at least one nucleic acid template and at least one energy source.

35. The *in vitro* synthesis system according to claim 1, comprising said at least one inhibitor, at least one nucleic acid template and at least one energy source.

36. The *in vitro* synthesis system according to claim 1, comprising at least one nucleic acid template and said at least two energy sources.

37. The *in vitro* synthesis system according to claim 1, comprising said at least one extract.

38. The *in vitro* synthesis system according to claim 1, comprising said at least one inhibitor.

39. A composition comprising one or more components selected from the group consisting of:

at least one extract from a cell having reduced activity of at least one enzyme that catalyzes hydrolysis of high energy phosphate bonds or hydrolysis or formation of phosphodiester bonds;

at least one inhibitor of at least one enzyme that catalyzes hydrolysis of high energy phosphate bonds or hydrolysis or formation of phosphodiester bonds; and

at least two energy sources providing chemical energy for synthesis.

40. A composition comprising one or more components selected from the group consisting of:

at least one extract from a cell having reduced activity of at least one enzyme selected from the group consisting of a nuclease, a polymerase and a phosphatase;

at least one inhibitor of at least one enzyme selected from the group consisting of a nuclease, a polymerase and a phosphatase; and

at least two energy sources providing chemical energy for synthesis.

41. A kit for *in vitro* synthesis comprising one or more of the components selected from the group consisting of:

at least one extract from a cell having reduced activity of at least one enzyme selected from the group consisting of a nuclease, a polymerase and a phosphatase;

at least one inhibitor of at least one enzyme selected from the group consisting of a nuclease, a polymerase and a phosphatase; and

at least two energy sources providing chemical energy for synthesis.

42. The kit according to claim 41, comprising one or more of the components selected from the group consisting of:

- at least one inhibitor of RecBCD;
- at least one cell mutated at at least one gene selected from the group consisting of a nuclease, a polymerase and a phosphatase;
- at least one extract of a cell mutated at at least one gene selected from the group consisting of a nuclease, a polymerase and a phosphatase;
- an inhibitor of at least one enzyme selected from the group consisting of a nuclease, a polymerase and a phosphatase;
- at least one energy source for synthesis; and
- a medium for growing said at least one cell.

43. A method for producing protein or nucleic acid from a nucleic acid template in an *in vitro* system comprising:

- contacting said template with at least one component selected from the group consisting of:
  - at least one extract from a cell having reduced activity of at least one enzyme that catalyzes hydrolysis of high energy phosphate bonds or hydrolysis or formation of phosphodiester bonds;
  - at least one inhibitor of at least one enzyme that catalyzes hydrolysis of high energy phosphate bonds or hydrolysis or formation of phosphodiester bonds; and
  - at least two energy sources providing chemical energy for synthesis,
- to form a mixture; and
- incubating said mixture under conditions sufficient to produce at least one protein encoded by said template.

44. The method according to claim 43, wherein the at least one enzyme is selected from the group consisting of OmpT, RNase E, alkaline phosphatase and endonuclease I.

45. The method according to claim 43, wherein the inhibitor is a Gam.

46. The method according to claim 41, wherein each of the at least two energy sources generates or regenerates high energy triphosphate compounds for protein synthesis.

47. The method according to claim 46, wherein the at least two energy sources are selected from the group consisting of pyruvate, phosphoenolpyruvate (PEP), carbamoyl phosphate, acetyl phosphate, creatine phosphate, phosphopyruvate, glyceraldehyde-3-phosphate and glucose-6-phosphate.

48. The method according to claim 43, wherein said enzyme is selected from the group consisting of a nuclease, a phosphatase and a polymerase.

49. A method for constructing an *in vitro* synthesis system, said method comprising:

obtaining at least one cell extract;

mixing the cell extract with one or more components selected from the group consisting of:

at least one inhibitor of at least one enzyme that catalyzes hydrolysis of high energy phosphate bonds or hydrolysis or formation of phosphodiester bonds; and

at least two energy sources providing chemical energy for synthesis.



50. The method according to claim 49, wherein said at least one enzyme is selected from the group consisting of a nuclease, a phosphatase and a polymerase.

51. A composition comprising one or more components selected from the group consisting of:

i) at least one extract from a cell having reduced activity of at least one enzyme that catalyzes hydrolysis of high energy phosphate bonds or hydrolysis or formation of phosphodiester bonds,

ii) at least one inhibitor of at least one enzyme that catalyzes hydrolysis of high energy phosphate bonds or hydrolysis or formation of phosphodiester bonds, and

iii) at least two energy sources providing chemical energy for synthesis; and

at least one nucleic acid template in the presence of at least a partial synthesis product of said template.

52. The composition according to claim 51, wherein the product is a nucleic acid product.

53. The composition according to claim 52, wherein the nucleic acid product is a DNA.

54. The composition according to claim 52, wherein the nucleic acid product is a RNA.